In the Claims

Please cancel claims 1-58.

Claims 59-108 are currently pending.

Remarks

Claims:

In the parent application, claims 1-58, as filed, were elected in response to a Restriction Requirement dated October 26, 1999. Accordingly, claims 1-58, having been already prosecuted in the parent application, are cancelled herewith. Currently pending claims 59-108 were deemed to be one invention according to the Restriction Requirement in the parent case.

Specification:

Applicants herewith introduce amendments made to the specification during the prosecution of the parent case.

Some of the foregoing amendments merely embody the correction of figure descriptions in order to make the specification consistent with the format of the formal drawings filed herewith.

Tables 1, 2, 3, 5, 6, 10, 11, 13 and 14, as well as other sections of the specification, were amended in order to introduce SEQ ID NO: for each nucleic acid sequence.

Tables 2, 3, 4, 5, and 11 were replaced, in part, to improve clarity and correct a few minor typographical errors without introduction of new matter.

Table 2 was replaced, in part, to correct the title. Support for this amendment can be found in the footnote.

Table 3 was replaced, in part, to correct the heading for column 3 by substituting "No CpG Motifs" with "No. CpG motifs". In addition, the singly underlined CG dinucleotides in footnotes 2 and 3 were replaced with doubly underlined CG dinucleotides so that all underlining is double.

Table 4 was replaced to change nomenclature as follows: In column 1, "pHIS20-S(ad)" was replaced with --pHIS40-S(ad)--; "pHIS36-S(ad)" was replaced with --pHIS64-S(ad)--; "pHIS72-S(ad)" was replaced with --pHIS128-S(ad)--; and "pHIS108-S(ad)" was replaced with --pHIS192-S(ad)--. In column 2, "pHIS-20" was replaced with --pHIS-40--; "pHIS-36" was replaced with --pHIS-64--; "pHIS-72" was replaced with --pHIS-128--; and

"pHIS-108" was replaced with --pHIS-192--. These corrections in nomenclature are supported at page 40, lines 10-23, as well as in Table 3.

Table 5 was replaced, in part, for clarity. The replacement Table is in a larger font for clarity, which necessitates the addition of a third page to accommodate the entire table.

Table 11 was replaced, in part, to insert "CpG" in the title between "good" and "Oligo 1619". There was no change in any of the sequence information in the table.

Table 14 was replaced, in part, to correct a nucleic acid sequence in footnote 2. Specifically, the sequence of ODN 1619 was incorrectly listed. Support for the correct sequence of ODN 1619 and this amendment can be found in Tables 11 and 13.

No new matter has been added by the foregoing amendments. If the Examiner has any questions or comments, he/she is respectfully requested to contact Applicants' representative at the number listed below.

Respectfully submitted,

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Docket No. C1039/7057
September 26, 2001
xndd

APPENDIX A

MARKED-UP SPECIFICATION

Please amend the specification as follows:

Please insert on page 1, line 3, after the title of the invention and prior to the section entitled Technical Field the following text:

Related Applications

This application is a divisional of U.S. non-provisional patent application serial no. 09/082,649, filed May 20, 1998, now allowed, which claims priority to U.S. provisional patent application serial no. 60/047,209, filed May 20, 1998 and U.S. provisional patent application serial no. 60/047,233, filed May 20, 1997.

Please note that the underlining of sequences in the proceeding marked-up specification does not indicate a change to the text, but rather reflects underlining of such sequences as present in the originally filed specification. Accordingly, no changes to sequences have been introduced by this amendment. In order to facilitate the identification of amendments to the specification, such amendments have also been highlighted as well as underlined or bracketed.

Please re-write the paragraph starting on page 5, line 13, as follows:

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B [1 is a] are schematic diagrams of the construction of pUK21-A1.

Figures 2A and 2B [2 is a] are schematic diagrams of the construction of pUK21-A2.

Figures 3A and 3B [3 is a] are schematic diagrams of the construction of pUK21-A.

Figures 4A and 4B [4 is a] are schematic diagrams of the construction of pMAS.

Please re-write the paragraph beginning on page 6, line 1, as follows:

Figure 6: Synthetic ODN cannot be mixed with DNA vaccine due to interference with expression from plasmid. The figure shows the effect of adding S-ODN to plasmid DNA expressing reporter gene or antigen. ODN 1826 (10 or 100 μg) was added to DNA constructs (10 μg) encoding hepatitis B surface antigen (HBsAg) (pCMV-S, [Figure 6A] top panel) or luciferase (pCMV-luc, [Figure 6B] bottom panel) DNA prior to intramuscular (IM) injection into mice. There was an ODN dose-dependent reduction in the induction of antibodies against HBsAg (anti-HBs, end-point dilution titers at 4 wk) by the pCMV-S DNA ([Figure 6A] top panel) and in the amount of luciferase expressed in relative light units per sec per mg protein

(RLU/sec/mg protein at 3 days) from the pCMV-luc DNA ([Figure 6B] bottom panel). This suggests that the lower humoral response with DNA vaccine plus ODN was due to decreased antigen expression. Each bar represents the mean of values derived from 10 animals ([Figure 6A] top panel) or 10 muscles ([Figure 6B] bottom panel) and[s] vertical lines represent the SEM. Numbers [superimposed on] below the bars indicate proportion of animals responding to the DNA vaccine ([Figure 6A] top panel); all muscles injected with pCMV-luc expressed luciferase ([Figure 6B] bottom panel).

Please re-write the paragraph beginning on page 6, line 13, as follows:

Figure 7: Interference of ODN with pDNA due to backbone and sequence. The figure shows the interference of ODN with plasmid DNA depends on backbone and sequence. Luciferase activity (RLU/sec/mg protein) in mouse muscles 3 days after they were injected with 10 µg ~~pCMV-luc DNA to which had been added no ODN~~ (none = white bar) or 100 µg of an ODN, which had one of three backbones: phosphorothioate (S = [black] left slanted bars: 1628, 1826, 1911, 1982, 2001 and 2017), phosphodiester (O = [pale grey] thick left slanted bar: 2061), or a phosphorothioate-phosphodiester chimera (SOS = [dark grey] right slanted bars: 1585, 1844, 1972, 1980, 1981, 2018, 2021, 2022, 2023 and 2042). Three S-ODN (1911, 1982 and 2017) and two SOS-ODN (1972 and 2042) did not contain any immunostimulatory CpG motifs. One S-ODN (1628) and three SOS-ODN (1585, 1972, 1981) had poly-G ends and one SOS-ODN (2042) had a poly-G center. The (*) indicates ODN of identical sequence but different backbone: 1826 (S-ODN), 1980 (SOS-ODN) and 2061 (O-ODN). All S-ODN (both CpG and non-CpG) resulted in decreased luciferase activity whereas SOS-ODN did not unless they had poly-G sequences.

Please re-write the paragraph beginning on page 6, line 25, as follows:

Figure 8: Temporal and spatial separation of CpG ODN and plasmid DNA. The figure shows the effect of temporal or spatial separation of plasmid DNA and S-ODN on gene expression. Luciferase activity (RLU/sec/mg protein) in mouse muscles 3 or 14 days after they were injected with 10 µg pCMV-luc DNA. Some animals also received 10 µg CpG-S ODN which was mixed with the DNA vaccine or was given at the same time but at a different site, or was given 4 days prior to or 7 days after the DNA vaccine. Only when the ODN was mixed directly with the DNA vaccine did it interfere with gene expression.

Please re-write the paragraph beginning on page 7, line 6, as follows:

Figure 9: Immunization of BALB/c mice with CpG-optimized DNA vaccines. The figure shows the enhancement of *in vivo* immune effects with optimized DNA vaccines. Mice were injected with 10 µg of pUK-S [(black bars)], pMAS-S [(white bars)], pMCG16-S [(pale grey bars)] or pMCG50-S [(dark grey bars)] plasmid DNA bilaterally (50 µl at 0.1 mg/ml in saline) into the TA muscle. [Figure 9A] The top panel shows the anti-HBs antibody response at 6 weeks (detected as described in methods). Bars represent the group means (n=5) for ELISA end-point dilution titers (performed in triplicate), and vertical lines represent the standard errors of the mean. The numbers on the bars indicate the ratio of IgG2a:IgG1 antibodies at 4 weeks, as determined in separate assays (also in triplicate) using pooled plasma. [Figure 9B] The bottom panel shows the cytotoxic T lymphocyte activity in specifically restimulated (5 d) splenocytes taken from mice 8 wk after DNA immunization. Bars represent the group means (n=3) for % specific lysis (performed in triplicate) at an ~~effector:target (E:T) ratio of 10:1, dots represent the individual~~ values. Non-specific lytic activity determined with non-antigen-presenting target cells, which never exceeds 10%, has been subtracted from values with HBsAg-expressing target cells to obtain % specific lysis values.

Please re-write the paragraph beginning on page 7, line 19, as follows:

Figure 10 shows induction of a Th2-like response by a CpG-N motif and inhibition of the Th1-like response induced by a CpG-S motif. Anti-HBs antibody titers (IgG1 and IgG2a subclasses) in BALB/c mice 12 weeks after IM immunization with recombinant HBsAg, which was given alone (none) or with 10 µg stimulatory ODN (1826), 10 µg of neutralizing ODN (1631, CGCGCGCGCGCGCGCGCGCG (SEQ ID NO:22) 1984, TCCATGCCGTTCCTGCCGTT (SEQ ID NO:78); or 2010 GCGGCGGGCGGCGCGCGCCC (SEQ ID NO:75); CpG dinucleotides are underlined for clarity) or with 10 μg stimulatory ODN + 10 μg neutralizing ODN. To improve nuclease resistance for these *in vivo* experiments, all ODN were phosphorothioate-modified. Each bar represents the group mean (n=10 for none; n=15 for #1826 and n=5 for all other groups) for anti-HBs antibody titers as determined by end-point dilution ELISA assay. [Black] Hatched portions of bars indicate antibodies of IgG1 subclass (Th2-like) and [grey] white portions indicate IgG2a subclass (Thl-like). The numbers above each bar indicate the IgG2a/IgG1 ratio where a ratio >1 [than] indicates a predominantly Thl-like response and a ratio <1 indicates a predominantly Th2-like response (a value of 0 indicates a complete absence of IgG2a antibodies).

Please re-write paragraph beginning on page 8, line 5, as follows:

Figure 11 shows enhancement of *in vivo* immune effects with optimized DNA vaccines. Mice were injected with 10 µg of pUK-S ([black] white bars), pMAS-S ([white] right slanted bars), pMCG16-S ([pale grey] thin right slanted bars) or pMCG50-S ([dark grey] left slanted bars) plasmid DNA bilaterally (50 µl at 0. 1 mg/ml in saline) into the TA muscle. Panel A: The anti-HBs antibody response at 6 weeks (detected as described in methods). Bars represent the group means (n=5) for ELISA end-point dilution titers (performed in triplicate), and vertical lines represent the standard errors of the mean. The numbers on the bars indicate the ratio of IgG2a:IgG1 antibodies at 4 weeks, as determined in separate assays (also in triplicate) using pooled plasma. Panel B: Cytotoxic T lymphocyte activity in specifically restimulated (5 d) splenocytes taken from mice 8 wk after DNA immunization. Bars represent the group means (n=3) for % specific lysis (performed in triplicate) at an effector: target (E:T) ratio of 10:1, dots represent the individual values. Non-specific lytic activity determined with non-antigen-presenting target cells, which never exceeds 10%, has been subtracted from values with HBsAg-expressing target cells to obtain % specific lysis values.

Please re-write the paragraph beginning on page 35, line 8, as follows:

(i) Insertion of the CMV (human cytomegalovirus) major intermediate early promoter/enhancer region

The CMV promoter (from pcDNA3 position 209 to 863) was amplified by PCR using 30 ng pcDNA3 as a template. The forward PCR primer 5'CGT GGA TAT CCG ATG TAC GGG CCA GAT AT 3'(SEQ ID NO:4) introduced an EcoRV site, and the reverse PCR primer 5' AGT CGC GGC CGC AAT TTC GAT AAG CCA GTA AG 3'(SEQ ID NO:5) introduced a *Not*I site. After digestion with EcoRV and *Not*I, a 0.7 kb PCR fragment containing the CMV promoter was purified and inserted into the pUK21 polylinker between XbaI and *Not*I sites. The *Xba*I sticky end of pUK21 was filled in with the large fragment of T4 DNA polymerase after digestion to create a blunt end. The inserted CMV promoter was confirmed by sequencing. The resulting plasmid was pUK21-Al (Figures 1A and 1B).

Please re-write the paragraph beginning on page 35, line 19, as follows:

(ii) Insertion of the BGH polyA (bovine growth hormone polyadenylation signal)

BGH polyA (from pcDNA3 position 1018 to 1249) was amplified by PCR using pcDNA3 as template. The forward PCR primer 5' ATT CTC GAG TCT AGA CTA GAG CTC GCT

GAT CAG CC 3' (SEQ ID NO:6) introduced *Xho*I and *Xba*I sites, and the reverse PCR primer 5' ATT AGG CCT TCC CCA GCA TGC CTG CTA TT 3' (SEQ ID NO:7) introduced a *Stu*I site. After digestion with *Xho*I and *Stu*I, the 0.2 kb PCR fragment containing the BGH polyA was purified, and ligated with the 3.7 kb *Xho*I-*Stu*I fragment of pUK21-Al. The inserted BGH polyA was confirmed by sequencing. The resulting plasmid was pUK21-A2 (Figures 2A and 2B).

Please re-write the paragraph beginning on page 36, line 24, as follows:

(i) Insertion of the fl origin of replication region

The fl origin and two unique restriction enzyme sites (*Dra*I and *Apa*I) were introduced into pUK21-A2 for later vector construction. fl origin (from pcDNA3 position 1313 to 1729) was amplified by PCR using pcDNA3 as template. The forward PCR primer 5' TAT AGG CCC ~~TAT TTT AAA CGC GCC CTG TAG CGG CGC~~ A 3' (SEQ ID NO:8) introduced *Eco*O109I and *Dra*I sites, and the reverse PCR primer 5' CTA TGG CGC CTT GGG CCC AAT TTT TGT TAA ATC AGC TC 3' (SEQ ID NO:9) introduced *Nar*I and *Apa*I site. After digestion with *Nar*I and *Eco*O109I, the 0.4 kb PCR fragment containing the fl origin was purified and ligated with the 3.3 kb *NarI-Eco*O109I fragment of pUK21-A2, resulting in pUK21-A (Figures 3A and 3B).

Please re-write the paragraph beginning on page 38, line 22, as follows:

(iii) Replacement of the fl origin with unique restriction enzyme sites

Oligonucleotides 5' AAA TTC GAA AGT ACT GGA CCT GTT AAC A 3' (SEQ ID NO:10) and its complementary strand 5' CGT GTT AAC AGG TCC AGT ACT TTC GAA TTT 3' (SEQ ID NO:11) were synthesized, and 5'-phosphorylated. Annealing of these two phosphorylated oligos resulted in 28 base pair double-stranded DNA containing three unique restriction enzyme sites (*Sca*I, *Ava*II, *Hpa*I), one sticky end and one blunt end. Replacing the 0.4 kb *Nar*I-*Dra*I fragment of pUK21-B with this double-stranded DNA fragment resulted in the universal vector pMAS for DNA vaccine development (Figures 4A and 4B and 5).

Please re-write the paragraph beginning on page 44, line 11, as follows:

In contrast to the success with protein antigens, attempts to augment immune responses induced by a HBsAg-expressing DNA vaccine by the addition of CpG-S ODN 1826 failed. Surprisingly, the immune responses decreased with the addition of CpG-S ODN in a dose-dependent manner (Figure 6[a], top panel). Addition of ODN #1826 to a luciferase reporter

gene construct (pCMV-luc, Davis *et al.,* 1993b) resulted in a dose-dependent decrease in luciferase expression (Figure 6[b], bottom panel). This indicates that the negative effects of the CpG-S ODN on the DNA vaccine were due to reduced gene expression rather than an effect on the immune response against the gene product.

Please re-write the paragraph beginning on page 48, line 15, as follows:
Next, different numbers of CpG-S motifs were inserted into the vector by allowing self-ligation of a 20bp DNA fragment with the sequence 5' GACTCCATGACGTTCCTGACGTTTCCATGACGTTCCTGACGTTG 3'(SEQ ID NO:[22] 12) with a complementary strand and inserting different numbers of copies into the *Ava*II site of pMAS. Recombinant clones were screened and the two vectors were chosen for further testing with 16 and 50 CpG-S motifs, and named pMCG16 and pMCG50 respectively.

Please re-write the paragraph beginning on page 51, line 16, as follows:
When tested for their ability to induce cytokine (IL-6 and IL-12) secretion from cultured spleen cells, we found that the pMAS-S, pMCG16-S and pMCG50-S vectors had significantly enhanced immune stimulatory activity compared to pUK-S. When used as a DNA vaccine, the anti-HBs response at 4 and 6 weeks was substantially stronger with DNA vaccines from which CpG-N motifs had been deleted, and even more so when 16 CpG-S motifs had been inserted. The vector with 50 CpG-S motifs, however, was less effective at inducing antibody production than that with 16 motifs. (Figure 11, panel A). Removal of CpG-N motifs and addition of CpG-S motifs resulted in a more than three-fold increase in the proportion of IgG2a relative to IgG1 anti-HBs antibodies, indicating an enhanced Th-1 response. This accentuated Th1 response also was demonstrated by the striking progressive increases in CTL responses induced by vectors from which CpG-N motifs were deleted and/or CpG-S motifs added (Figure 11, panel B).

Please re-write the paragraph beginning on page 53, line 20, as follows:
Based on our *in vitro* experiments we hypothesized that the presence of CpG-N motifs in DNA vaccines interferes with the induction of the desired immune response. Indeed, the present study demonstrates that elimination of CpG-N motifs from a DNA vaccine leads to improved induction of antibodies. By removing 52 of the CpG-N motifs from a DNA vaccine (45 were deleted and 7 turned into CpG-S motifs) the serologic response was more than doubled; by then adding an additional 16 CpG-S motifs, the response was enhanced

nearly 10 fold (Figure 11, panel A). Likewise, CTL responses were improved by removing CpG-N motifs and even more so by adding 16 or 50 CpG-S motifs (Figure 11, panel B). These increased responses are especially notable in view of the fact that the total number of CpG dinucleotides in the mutated vaccines is considerably below the original number.

Please re-write the paragraph beginning on page 54, line 2, as follows:
The finding that the vector with 50 CpG-S motifs was inferior to that with 16 motifs for induction of humoral immunity was unexpected, and may be secondary to CpG-induced production of type I interferons, and subsequent reduction in the amount of antigen expressed. The decreased antibody response induced by pMCG50-S seems unlikely to be explained by vector instability since this vector gave the best CTL responses (Figure 11, panel B). Although the pMCG50-S vector was slightly larger than pMCG16-S, the 10 μg dose still contained 93% as many plasmid copies as it did pMCG16-S, so lower copy number is unlikely to account for the reduced antibody levels. The current generation of DNA vaccines are quite effective in mice, but much less effective in primates (Davis, H.L., *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:7213-7218 (1996); Letvin, N.L., *et al., Proc. Natl. Acad. Sci. USA,* 94:9378-9383 (1997); Fuller, D.H., *et al., J Med. Primatol.*, 25:236-241 (1996); Lu, S., *et al., J. Virol.*, 70:3978-3991 (1996); Liu, M.A., *et al., Vaccine,* 15:909-919 (1997); Prince, A.M., *et al., Vaccine,* 15:9196-919 (1997); Gramzinski, R.A., *et al., Molec. Med.,* 4:109-119 (1998)). Our present results indicate that attaining the full clinical potential of DNA vaccines will require using engineered vectors in which CpG-N motifs have been deleted, and CpG-S motifs added.

Please re-write Table 1, beginning on page 56, line 22, as follows:

Table 1.

Primers used for site-directed mutagenesis.

Mutated nucleotides are underlined. Restriction enzyme sites for cloning, are indicated in bold.

Forward primers:

Mu-0F		5' GTC**TCTAGACAGCCACTG**GTAACAGGATT 3' (845) (SEQ ID NO:23)
Mu-1F	(1144)	5' GTCGTTGTGTCGTCAAGTCAGCGTAATGC 3' (1172) (SEQ ID NO:24)
Mu-2F	(1285)	5' TCGTTTCTGTAATGAAGGAG 3' (1304) (SEQ ID NO:25)
Mu-3F	(1315)	5' AAGGCAGTTCCATAGGATGG 3' (1334) (SEQ ID NO:26)
Mu-(4+5)F	(1348)	5' TCGATCTGCGATTCCAACTCGTCCAACATCAATAC 3' (1382) (SEQ ID NO:27)
Mu-6F	(1453)	5' TGGTGAGAATGGCAAAAGTT 3' (1472) (SEQ ID NO:28)
Mu-7F	(1548)	5' CATTATTCATTCGTGATTGCG 3' (1568) (SEQ ID NO:29)
Mu-8F	(1633)	5' ACGTCTCAGGAACACTGCCAGCGC 3' (1656) (SEQ ID NO:30)
Mu-9F	(1717)	5' AGGGATCGCAGTGGTGAGTA 3' (1736) (SEQ ID NO:31)
Mu-10F	(1759)	5' TATAAAATGCTTGATGGTCGG 3' (1779) (SEQ ID NO:32)
Mu-(11+12)F	(1777)	5' GGGAAGAGGCATAAATTCTGTCAGCCAGTTTAGTC 3' (1811) (SEQ ID NO:33)
Mu-13F	(1882)	5' TGGCTTCCCATACAAGCGAT 3' (1901) (SEQ ID NO:34)
Mu-14F	(1924)	5' TACATTATCGCGAGCCCATT 3' (1943) (SEQ ID NO:35)
Mu-15F	(1984)	5' TGGCCTCGACGTTTCCCGT 3' (2002) (SEQ ID NO:36)

Reverse primers:

Mu-0R		5' ATC**GAATTCAGGGCC**TCGTGATACGCCTA 3' (2160) (SEQ ID NO:37)
Mu-1R	(1163)	5' TGACTTGACGACACAACGACAGCTCATGACCAAAATCCC 3' (1125) (SEQ ID NO:38)
Mu-2R	(1304)	5' CTCCTTCATTACAGAAACGACTTTTTCAAAAATATGGTA 3' (1266) (SEQ ID NO:39)
Mu-3R	(1334)	5' CCATCCTATGGAACTGCCTTGGTGAGTTTTCTCCTTC 3' (1298) (SEQ ID NO:40)
Mu-(4+5)R	(1367)	5' GAGTTGGAATCGCAGATCGATACCAGGATCTTGC 3' (1334) (SEQ ID NO:41)
Mu-6R	(1472)	5' AACTTTTGCCATTCTCACCAGATTCAGTCGTCACTCA 3' (1436) (SEQ ID NO:42)
Mu-7R	(1568)	5' CGCAATCACGAATGAATAATGGTTTGGTTGATGCGAGTG 3' (1530) (SEQ ID NO:43)

Mu-8R	(1652)	5' TGGCAGTGTTCCTGAGACGTTTGCATTCGATTCCTGTT 3' (1615) (SEQ ID NO:44)
Mu-9R	(1736)	5' TACTCACCACTGCGATCCCTGGAAAAACAGCATTCCAG 3' (1736) (SEQ ID NO:45)
Mu-10R	(1779)	5' CCGACCATCAAGCATTTTATACGTACTCCTGATGATGCA 3' (1741) (SEQ ID NO:46)
Mu-(11+12)	(1796)	5' CAGAATTTATGCCTCTTCCCACCATCAAGCATTTTATAC 3' (1758) (SEQ ID NO:47)
Mu-13R	(1901)	5' ATCGCTTGTATGGGAAGCCAGATGCGCCAGAGTTGTTT 3' (1882) (SEQ ID NO:48)
Mu-14R	(1943)	5' AATGGGCTCGCGATAATGTAGGGCAATCAGGTGCGAC 3' (1907) (SEQ ID NO:49)
Mu-15R	(2002)	5' ACGGGAAACGTCGAGGCCACGATTAAATTCCAACATGG 5' (1965) (SEQ ID NO:50)

[(SEQ ID NO:23-50, respectively)]

Please re-write Table 2, beginning on page 59, line 1, as follows:

Table 2 *Nucleotide and amino acid sequences of the AlwNI-EcoO109I fragment* (SEQ ID NO:80)

kan(wt)	2180	AAGGGCCTCG	TGATACGCCT	ATTTTTATAG	GTTAATGTCA	TGGGGGGGGG	GGGGAAAGCC
kan(wt)	2120	ACGTTGTGTC	TCAAAATCTC	TGATGTTACA	TTGCACAAGA	TAAAAATATA	TCATCATGAA
kan(wt)	2060	CAATAAAACT	GTCTGCTTAC	ATAAACAGTA	ATACAAGGGG	TGTTATGAGC	CATATTCAAC
kan(mu)							
ORF						M S	H I Q
kan(wt)	2000	GGGAAACGTC	GAGGCCGCGA	TTAAATTCCA	ACATGGATGC	TGATTTATAT	GGGTATAAAT
kan(mu)			A				
ORF		R E T S	R P R	L N S	N M D A	D L Y	G Y K
kan(wt)	1940	GGGCTCGCGA	TAATGTCGGG	CAATCAGGTG	CGACAATCTA	TCGCTTGTAT	GGGAAGCCCG
kan(mu)			A				A
ORF		W A R D	N V G	Q S G	A T I Y	R L Y	G K P
kan(wt)	1880	ATGCGCCAGA	GTTGTTTCTG	AAACATGGCA	AAGGTAGCGT	TGCCAATGAT	GTTACAGATG
kan(mu)							
ORF		D A P E	L F L	K H G	K G S V	A N D	V T D
kan(wt)	1820	AGATGGTCAG	ACTAAACTGG	CTGACGGAAT	TTATGCCTCT	TCCGACCATC	AAGCATTTTA
kan(mu)				A		C	
ORF		E M V R	L N W	L T E	F M P L	P T I	K H F
kan(wt)	1760	TCCGTACTCC	TGATGATGCA	TGGTTACTCA	CCACTGCGAT	CCCCGGAAAA	ACAGCATTCC
~~kan(mu)~~		A				T	
ORF		I R T P	D D A	W L L	T T A I	P G K	T A F
kan(wt)	1700	AGGTATTAGA	AGAATATCCT	GATTCAGGTG	AAAATATTGT	TGATGCGCTG	GCAGTGTTCC
kan(mu)							
ORF		Q V L E	E Y P	D S G	E N I V	D A L	A V F
kan(wt)	1640	TGCGCCGGTT	GCATTCGATT	CCTGTTTGTA	ATTGTCCTTT	TAACAGCGAT	CGCGTATTTC
kan(mu)		A A A					
ORF		L R R L	H S I	P V C	N C P F	N S D	R V F
kan(wt)	1580	GTCTCGCTCA	GGCGCAATCA	CGAATGAATA	ACGGTTTGGT	TGATGCGAGT	GATTTTGATG
kan(mu)					T		
ORF		R L A Q	A Q S	R M N	N G L V	D A S	D F D
kan(wt)	1520	ACGAGCGTAA	TGGCTGGCCT	GTTGAACAAG	TCTGGAAAGA	AATGCATAAA	CTTTTGCCAT
kan(mu)							
ORF		D E R N	G W P	V E Q	V W K E	M H K	L L P
kan(wt)	1460	TCTCACCGGA	TTCAGTCGTC	ACTCATGGTG	ATTTCTCACT	TGATAACCTT	ATTTTTGACG
kan(mu)		A					
ORF		F S P D	S V V	T H G	D F S L	D N L	I F D
kan(wt)	1400	AGGGGAAATT	AATAGGTTGT	ATTGATGTTG	GACGAGTCGG	AATCGCAGAC	CGATACCAGG
kan(mu)					T	T	
ORF		E G K L	I G C	I D V	G R V G	I A D	R Y Q
kan(wt)	1340	ATCTTGCCAT	CCTATGGAAC	TGCCTCGGTG	AGTTTTCTCC	TTCATTACAG	AAACGGCTTT
kan(mu)				T			T
ORF		D L A I	L W N	C L G	E F S P	S L Q	K R L
kan(wt)	1280	TTCAAAAATA	TGGTATTGAT	AATCCTGATA	TGAATAAATT	GCAGTTTCAT	TTGATGCTCG
kan(mu)							
ORF		F Q K Y	G I D	N P D	M N K L	Q F H	L M L
kan(wt)	1220	ATGAGTTTTT	CTAATCAGAA	TTGGTTAATT	GGTTGTAACA	CTGGCAGAGC	ATTACGCTGA
kan(mu)							
ORF		D E F F					
kan(wt)	1160	CTTGACGGGA	CGGCGCAAGC	TCATGACCAA	AATCCCTTAA	CGTGAGTTTT	CGTTCCACTG
kan(mu)		AC	AA AC				
kan(wt)	1100	AGCGTCAGAC	CCCGTAGAAA	AGATCAAAGG	ATCTTCTTGA	GATCCTTTTT	TTCTGCGCGT
kan(wt)	1040	AATCTGCTGC	TTGCAAACAA	AAAAACCACC	GCTACCAGCG	GTGGTTTGTT	TGCCGGATCA
kan(wt)	980	AGAGCTACCA	ACTCTTTTTC	CGAAGGTAAC	TGGCTTCAGC	AGAGCGCAGA	TACCAAATAC
kan(wt)	920	TGTTCTTCTA	GTGTAGCCGT	AGTTAGGCCA	CCACTTCAAG	AACTCTGTAG	CACCGCCTAC
kan(wt)	860	ATACCTCGCT	CTGCTAATCC	TGTTACCAGT	GGCTGCTGCC		

Note: Mutated nucleotides are underlined. The *Alw*NI and *Eco*O109I sites are indicated in bold type. The nucleotide numbering scheme is the same as the backbone vector pUK21.

Please re-write Table 3, beginning on page 60, line 1, as follows:

Plasmid DNA Vectors Davis *et al.* (1998)

Table 3
Plasmids containing immunostimulatory CpG motifs

Plasmid	Backbone	[No] No. CpG Motifs	Species Specificity and ODN Equivalence of CpG-S Insert
pMCG-16	pMAS	16	mouse-specific CpG motif #1826[1]
pMCG-50	pMAS	50	
pMCG-100	pMAS	100	
pMCG-200	pMAS	200	
pHCG-30	pMAS	30	human-specific CpG motif - no ODN equivalent[2]
pHCG-50	pMAS	50	
pHCG-100	pMAS	100	
pHCG-200	pMAS	200	
pHIS-40	pMAS	40	human-specific CpG motif #2006[3]
pHIS-64	pMAS	64	
pHIS-128	pMAS	128	
pHIS-192	pMAS	192	

[1] sequence of 1826 is TCCATGACGTTCCTGACGTT (SEQ ID NO:51)

[2] sequence used as a source of CpG motifs is GACTTCGTGTCGTTCTTCTGTCGTCTTTAGCGCTTCTCCTGCGTGCGTCCCTTG (SEQ ID NO:14)

[3] sequence of 2006 is TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO:3)

Please re-write Table 4, beginning on page 61, line 1, as follows:

Table 4

Plasmids encoding hepatitis B surface antigen (derived from ayw or adw subtypes of HBV)

Plasmid	Backbone	Insert
pUK-S	pUK21-A2	HBV-S (ayw)
pUKAX-S	pUK21-AX*	HBV-S (ayw)
pMAS-S	pMAS	HBV-S (ayw)
pMCG16-S	pMCG-16	HBV-S (ayw)
pMCG50-S	pMCG-50	HBV-S (ayw)
pMCG100-S	pMCG-100	HBV-S (ayw)
pMCG200-S	pMCG-200	HBV-S (ayw)
pHCG30-S	pHCG-30	HBV-S (ayw)
~~pHCG50-S~~	~~pHCG-50~~	~~HBV-S (ayw)~~
pHCG100-S	pHCG-100	HBV-S (ayw)
pHCG200-S	pHCG-200	HBV-S (ayw)
[pHIS20-S(ad)] pHIS40-S(ad)	[pHIS-20] pHIS-40	HBV-S (adw2)
[pHIS36-S(ad)] pHIS64-S(ad)	[pHIS-36] pHIS-64	HBV-S (adw2)
[pHIS72-S(ad)] pHIS128-S(ad)	[pHIS-72] pHIS-128	HBV-S (adw2)
[pHIS108-S(ad)] pHIS192-S(ad)	[pHIS-108] pHIS-192	HBV-S (adw2)

*pUK21-AX was created by deleting f1 origin from pUK21-A

Please re-write Table 5, beginning on page 62, line 1, as follows:

Table 5 *Sequence comparison of pUK21-A2* <u>(SEQ ID NO:83)</u> *and pGT* <u>(SEQ ID NO:84)</u>.
75 point-mutations (indicated with *) in pUK21-A2 results in the gene therapy vector (pGT)

```
pUK21-A2(1)       GAATTCGAGC TCCCGGGTAC CATGGCATGC ATCGATAGAT CTCGAGTCTA GACTAGAGCT
pGT               GAATTCGAGC TCCCGGGTAC CATGGCATGC ATCGATAGAT CTCGAGTCTA GACTAGAGCT
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(61)      CGCTGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC
pGT               CGCTGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(121)     GTGCCTTCCT TGACCCTGGA AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA
pGT               GTGCCTTCCT TGACCCTGGA AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(181)     ATTGCATCGC ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC
pGT               ATTGCATCGC ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(241)     AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG GGGAAGGCCT CGGACTAGTG
pGT               AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG GGGAAGGCCT CGGACTAGTG
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(301)     GCGTAATCAT GGTCATAGCT GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC
pGT               CCGGAATCAT GGTCATAGCT GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC
                  *--*------ ---------- ---------- ---------- ---------- ----------
pUK21-A2(361)     AACATACGAG CCGCGGAAGC ATAAAGTGTA AAGCCTGGGG TGCCTAATGA GTGAGCTAAC
pGT               AACATCCGGG CCGCGGAAGC ATAAAGTGTA AAGCCTGGGG TGCCTAATGA GTGAGCTAAC
                  -----*--*- ---------- ---------- ---------- ---------- ----------
pUK21-A2(421)     TCACATTAAT TGCGTTGCGC TCACTGCCCG CTTTCCAGTC GGGAAACCTG TCGTGCCAGC
pGT               TCACATTAAT TCCGTTCCGC TCACTGCCCG CTTTCCAGTC GGGAAACCTG CCGTGCCAGC
                  ---------- -*----*--- ---------- ---------- ---------- *---------
pUK21-A2(481)     TGCATTAATG AATCGGCCAA CGCGCGGGGA GAGGCGGTTT GCGTATTGGG CGCTCTTCCG
pGT               TGCATTAATG AATCGGCCAA CGCGCGGGGA GAGCCGGTTT CCGTATTGGC CGCTCTTCCG
                  ---------- ---------- ---------- ---*------ *--------* ----------
pUK21-A2(541)     CTTCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC
pGT               CTTCCTCGCT CACTGACTCG CTGCGCTCGG TCGTTCGGCT GCGGCGAGCG GTATCAGCTC
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(601)     ACTCAAAGGC GGTAATACGG TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACATGT
pGT               ACTCAAAGGC GGTAATACGG TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACATGT
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(661)     GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC CGCGTTGCTG GCGTTTTTCC
pGT               GAGCAAAAGG CCAGCAAAAG GCCAGGAACC GTAAAAAGGC CGCGTTGCTG GCGTTTTTCC
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(721)     ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG CTCAAGTCAG AGGTGGCGAA
pGT               ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG CTCAAGTCAG AGGTGGCGAA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(781)     ACCCGACAGG ACTATAAAGA TACCAGGCGT TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC
pGT               ACCCGACAGG ACTATAAAGA TACCAGGCGT TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(841)     CTGTTCCGAC CCTGCCGCTT ACCGGATACC TGTCCGCCTT TCTCCCTTCG GGAAGCGTGG
pGT               CTGTTCCGAC CCTGCCGCTT ACCGGATACC TGTCCGCCTT TCTCCCTTCG GGAAGCGTGG
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(901)     CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC
pGT               CGCTTTCTCA TAGCTCACGC TGTAGGTATC TCAGTTCGGT GTAGGTCGTT CGCTCCAAGC
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(961)     TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACTATC
pGT               TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACTATC
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(1021)    GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT GGCAGCAGCC ACTGGTAACA
pGT               TGGGCTGTGT GCACGAACCC CCCGTTCAGC CCGACCGCTG CGCCTTATCC GGTAACTATC
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(1081)    GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT
pGT               GGATTAGCAG AGCGAGGTAT GTAGGCGGTG CTACAGAGTT CTTGAAGTGG TGGCCTAACT
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(1141)    ACGGCTACAC TAGAAGAACA GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG
pGT               ACGGCTACAC TAGAAGAACA GTATTTGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(1201)    GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT
pGT               GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(1261)    GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT
pGT               GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTTT
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(1321)    TTTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCACG TTAAGGGATT TTGGTCATGA
pGT               TTTCTACGGG GTCTGACGCT CAGTGGAACG AAAACTCACG TTAAGGGATT TTGGTCATGA
                  ---------- ---------- ---------- ---------- ---------- ----------
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pUK21-A2(1381)    GCTTGCGCCG TCCCGTCAAG TCAGCGTAAT GCTCTGCCAG TGTTACAACC AATTAACCAA
pGT               GCTTGCGCCG TCCCGTCAAG TCACCGGAAT GCTCTGCCAG TGTTACAACC AATTAACCAA
                  ---------- ---------- ---*--*--- ---------- ---------- ----------
pUK21-A2(1441)    TTCTGATTAG AAAAACTCAT CGAGCATCAA ATGAAACTGC AATTTATTCA TATCAGGATT
pGT               TTCTGATTAG AAAAACTCAT CCAGCATCAA ATGAAACTGC AATTTATTCA TATCAGGATT
                  ---------- ---------- -*-------- ---------- ---------- ----------
pUK21-A2(1501)    ATCAATACCA TATTTTTGAA AAAGCCGTTT CTGTAATGAA GGAGAAAACT CACCGAGGCA
pGT               ATCAATACCA TATTTTTGAA AAAGCCGTTT CTGTAATGAA GGAGAAAACT CACCGAGGCA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(1561)    GTTCCATAGG ATGGCAAGAT CCTGGTATCG GTCTGCGATT CCGACTCGTC CAACATCAAT
pGT               GTTCCATAGG ATGGCAAGAT CCTGGTATCG GTCTGCAATT CCGACTCGGC CAACATCAAT
                  ---------- ---------- ---------- ------*--- --------*- ----------
pUK21-A2(1621)    ACAACCTATT AATTTCCCCT CGTCAAAAAT AAGGTTATCA AGTGAGAAAT CACCATGAGT
pGT               ACAACCTATT AATTTCCCCT CATCAAAAAT AAGGTTATCA AGTGAGAAAT CACCATGAGT
                  ---------- ---------- -*-------- ---------- ---------- ----------
pUK21-A2(1681)    GACGACTGAA TCCGGTGAGA ATGGCAAAAG TTTATGCATT TCTTTCCAGA CTTGTTCAAC
pGT               AACTACTGAA TCCGGTGAGA ATGGCAAAAG TTTATGCATT TCTTTCCAGA CTTGTTCAAC
                  *--*------ ---------- ---------- ---------- ---------- ----------
pUK21-A2(1741)    AGGCCAGCCA TTACGCTCGT CATCAAAATC ACTCGCATCA ACCAAACCGT TATTCATTCG
pGT               AGGCCAGCCA TTACGCTCAT CATCAAAATC GGAAGCATCA ACCAAACCGT TATTCATTCG
                  ---------- --------*- ---------- ****------ ---------- ----------
pUK21-A2(1801)    TGATTGCGCC TGAGCGAGAC GAAATACGCG ATCGCTGTTA AAAGGACAAT TACAAACAGG
pGT               GGATTGAGCC TGAGCCAGAC GGAATACGCG GTCGCTGTTA AAAGGACAAT TACAAACAGG
                  *-----*--- -----*---- -*-------- *--------- ---------- ----------
pUK21-A2(1861)    AATCGAATGC AACCGGCGCA GGAACACTGC GAGCGGATCA ACAATATTTT CACCTGAATC
pGT               AATGGAATGC AACCGGCGGA GGAACACTGC CAGAGCATCA ACAATATTTT CACCTGAATC
                  ---*------ --------*- ---------- ---*------ ---------- ----------
pUK21-A2(1921)    AGGATATTCT TCTAATACCT GGAATGCTGT TTTTCCGGGG ATCGCAGTGG TGAGTAACCA
pGT               AGGATATTCT TCTAATACCT GGAATGCTGT TTTTCCGGGG ATAGCAGTGG TGAGTAACCA
                  ---------- ---------- ---------- ---------- --*------- ----------
pUK21-A2(1981)    TGCATCATCA GGAGTACGGA TAAAATGCTT GATGGTCGGA AGAGGCATAA ATTCCGTCAG
pGT               TGCATCATCA GGAGTACGGA TAAAATGCTT GATGGTCGGA AGAGGCATAA ATTCCGTCAG
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(2041)    CCAGTTTAGT CTGACCATCT CATCTGTAAC ATCATTGGCA ACGCTACCTT TGCCATGTTT
pGT               CCAGTTTAGT CTGACCATCT CATCTGTAAC ATCATTGGCA ACGCTACCTT TGCCATGTTT
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(2101)    CAGAAACAAC TCTGGCGCAT CGGGCTTCCC ATACAAGCGA TAGATTGTCG CACCTGATTG
pGT               CAGAAACAAC TCCGGCGCGT CGGGCTTCCC ATACAAGCGG TAGATTGTAG CACCTGATTG
                  ---------- --*-----*- ---------- ---------* --------*- ----------
pUK21-A2(2161)    CCCGACATTA TCGCGAGCCC ATTTATACCC ATATAAATCA GCATCCATGT TGGAATTTAA
pGT               CCCGACATTA TCGCGAGCCC ATTTATACCC ATATAAATCA GCATCCATGT TGGAATTTAA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(2221)    TCGCGGCCTC GACGTTTCCC GTTGAATATG GCTCATAACA CCCCTTGTAT TACTGTTTAT
pGT               TCGCGGCCTG GAGGTTTCCC GTTGAATATG GCTCATAACA CCCCTTGTAT TACTGTTTAT
                  ---------* --*------- ---------- ---------- ---------- ----------
pUK21-A2(2281)    GTAAGCAGAC AGTTTTATTG TTCATGATGA TATATTTTTA TCTTGTGCAA TGTAACATCA
pGT               GTAAGCAGAC AGTTTTATTG TTCATGATGA TATATTTTTA TCTTGTGCAA TGTAACATCA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(2341)    GAGATTTTGA GACACAACGT GGCTTTCCCC CCCCCCCCCA TGACATTAAC CTATAAAAAT
pGT               GAGATTTTGA GACACACCGG GGCTTTCCCC CCCCCCCCCA TGACATTAAC CTATAAAAAT
                  ---------- ------*--* ---------- ---------- ---------- ----------
pUK21-A2(2401)    AGGCGTATCA CGAGGCCCTT TCGTCTCGCG CGTTTCGGTG ATGACGGTGA AAACCTCTGA
pGT               AGCCGTATCC CGAGGCCCTT CCGTCTCGCG CGTTCCGGTG ATGCCGGTGA AAACCTCTGA
                  --*------* ---------- *--------- ----*----- ---*------ ----------
pUK21-A2(2461)    CACATGCAGC TCCCGGAGAC GGTCACAGCT TGTCTGTAAG CGGATGCCGG GAGCAGACAA
pGT               CACATGCAGC TCCCGGAGAC GGTCACAGCT TGTCTGTAAG CGGATGCCGG GAGCAGACAA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(2521)    GCCCGTCAGG GCGCGTCAGC GGGTGTTGGC GGGTGTCGGG GCTGGCTTAA CTATGCGGCA
pGT               GCCCGTCAGG GCGCGTCAGC GGGTGTTGGC GGGTGTCGGG GCTGGCTTAA CTATGCGGCA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(2581)    TCAGAGCAGA TTGTACTGAG AGTGCACCAT AAAATTGTAA ACGTTAATAT TTTGTTAAAA
pGT               TCAGAGCAGA TTGTACTGAG AGTGCACCAT AAAATTGTAA CCGTTAATAT TTTGTTAAAA
                  ---------- ---------- ---------- ---------- *--------- ----------
pUK21-A2(2641)    TTCGCGTTAA ATTTTTGTTA AATCAGCTCA TTTTTTAACC AATAGACCGA AATCGGCAAA
pGT               TTCGCGTTAA ATTTTTGTTA AATCAGCTCA TTTTTTAACC AATAGACCGA AATCGGCAAA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(2701)    ATCCCTTATA AATCAAAAGA ATAGCCCGAG ATAGAGTTGA GTGTTGTTCC AGTTTGGAAC
pGT               ATCCCTTATA AATCAAAAGA ATAGCCCGAG ATAGAGTTGA GTGTTGTTCC AGTTTGGAAC
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(2761)    AAGAGTCCAC TATTAAAGAA CGTGGACTCC AACGTCAAAG GGCGAAAAAC CGTCTATCAG
pGT               AAGAGTCCAC TATTAAAGAC CGTGGACTCC ACCGTCAAAG GCCGAAAAAC CGTCTATCAG
                  ---------- ---------* ---------- -*-------- -*-------- ----------
```

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pUK21-A2(2821)    GGCGATGGCC CACCCCGATT TAGAGCTTGA CGGGGAAAGC CGGCGAACGT GGCGAGAAAG
pGT               GCCGATGGCC CACCCCGATT TAGAGCTTGA CGGGGAAAGC CGGCGCGCGT GCCGAGAAAG
                  -*-------- ---------- ---------- ---------- -----**--- -*--------
pUK21-A2(2881)    GAAGGGAAGA AAGCGAAAGG AGCGGGCGCT AAGGCGCTGG CAAGTGTAGC GGTCACGCTG
pGT               GAAGGGAAGA AACCGAAAGG AGCGGCCGCT AAGCCGCTGG CAAGTGTAGC GGTCCCGCTG
                  ---------- --*------- -----*---- ---*------ ---------- ----*-----
pUK21-A2(2941)    CGCGTAACCA CCACACCCGC CGCGCTTAAT GCGCCGCTAC AGGGCGCGTA CTATGGTTGC
pGT               CGCGTAACCA CCACACCCGC CGCGCTTAAT CCGCCGCTAC AGGGCGCGTA CTATGGTTGC
                  ---------- ---------- ---------- *--------- ---------- ----------
pUK21-A2(3001)    TTTGACGTAT GCGGTGTGAA ATACCGCACA GATGCGTAAG GAGAAAATAC CGCATCAGGC
pGT               TTTGCCGTAT GCGGTGTGAA ATACCGCACA GATCCGTAAG GAGAAAATAC CGCATCAGCC
                  ----*----- ---------- ---------- ---*------ ---------- --------*-
pUK21-A2(3061)    GCCATTCGCC ATTCAGGCTG CGCAACTGTT GGGAAGGGCG ATCGGTGCGG GCCTCTTCGC
pGT               GCCATCCGCC ATTCAGGCTC CGCAACTGTT GGGAAGGCCG ATCGGTGCGG GCCTCTCCGC
                  -----*---- ---------* ---------- -------*-- ---------- ------*---
pUK21-A2(3121)    TATTACGCCA GCTGGCGAAA GGGGGATGTG CTGCAAGGCG ATTAAGTTGG GTAACGCCAG
pGT               TATTCCGCCA GCTGCCGAAA GGGGGATGTG CTGCAAGCCG ATTAAGTTGG GTACCGCCAG
                  ----*----- ----*----- ---------- -------*-- ---------- ---*------
pUK21-A2(3181)    GGTTTTCCCA GTCACGACGT TGTAAAACGA CGGCCAGTGA ATTGTAATAC GACTCACTAT
pGT               GGTTTTCCCA GTCACGGCGG TGTAAACCGA CGGCCAGTGA ATTGTAATCC GACTCACTAT
                  ---------- ------*--* ------*--- ---------- --------*- ----------
pUK21-A2(3241)    AGGGCGAATT GGGGATCGAT CCACTAGTTC TAGATCCGAT GTACGGGCCA GATATACGCG
pGT               AGGCCGAATT GGGGACCGAT CCACTAGTTC TAGATCCGAT GTACGGGCCA GATATACGCG
                  ---*------ -----*---- ---------- ---------- ---------- ----------
pUK21-A2(3301)    TTGACATTGA TTATTGACTA GTTATTAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG
pGT               TTGACATTGA TTATTGACTA GTTATTAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3361)    TTGACATTGA TTATTGACTA GTTATTAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG
pGT               TTGACATTGA TTATTGACTA GTTATTAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3421)    CAACGACCCC CGCCCATTGA CGTCAATAAT GACGTATGTT CCCATAGTAA CGCCAATAGG
pGT               CAACGACCCC CGCCCATTGA CGTCAATAAT GACGTATGTT CCCATAGTAA CGCCAATAGG
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3481)    GACTTTCCAT TGACGTCAAT GGGTGGAGTA TTTACGGTAA ACTGCCCACT TGGCAGTACA
pGT               GACTTTCCAT TGACGTCAAT GGGTGGAGTA TTTACGGTAA ACTGCCCACT TGGCAGTACA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3541)    TCAAGTGTAT CATATGCCAA GTACGCCCCC TATTGACGTC AATGACGGTA AATGGCCCGC
pGT               TCAAGTGTAT CATATGCCAA GTACGCCCCC TATTGACGTC AATGACGGTA AATGGCCCGC
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3601)    CTGGCATTAT GCCCAGTACA TGACCTTATG GGACTTTCCT ACTTGGCAGT ACATCTACGT
pGT               CTGGCATTAT GCCCAGTACA TGACCTTATG GGACTTTCCT ACTTGGCAGT ACATCTACGT
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3661)    ATTAGTCATC GCTATTACCA TGGTGATGCG GTTTTGGCAG TACATCAATG GGCGTGGATA
pGT               ATTAGTCATC GCTATTACCA TGGTGATGCG GTTTTGGCAG TACATCAATG GGCGTGGATA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3721)    GCGGTTTGAC TCACGGGGAT TTCCAAGTCT CCACCCCATT GACGTCAATG GGAGTTTGTT
pGT               GCGGTTTGAC TCACGGGGAT TTCCAAGTCT CCACCCCATT GACGTCAATG GGAGTTTGTT
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3781)    TTGGCACCAA AATCAACGGG ACTTTCCAAA ATGTCGTAAC AACTCCGCCC CATTGACGCA
pGT               TTGGCACCAA AATCAACGGG ACTTTCCAAA ATGTCGTAAC AACTCCGCCC CATTGACGCA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3841)    AATGGGCGGT AGGCGTGTAC GGTGGGAGGT CTATATAAGC AGAGCTCTCT GGCTAACTAG
pGT               AATGGGCGGT AGGCGTGTAC GGTGGGAGGT CTATATAAGC AGAGCTCTCT GGCTAACTAG
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3901)    AGAACCCACT GCTTACTGGC TTATCGAAAT TGCGGCCGCC ACGGCGATAT CGGATCCATA
pGT               AGAACCCACT GCTTACTGGC TTATCGAAAT TGCGGCCGCC ACGGCGATAT CGGATCCATA
                  ---------- ---------- ---------- ---------- ---------- ----------
pUK21-A2(3961)    TGACGTCGAC GCGTCTGCAG AAGCTTC
pGT               TGACGTCGAC GCGTCTGCAG AAGCTTC
                  ---------- ---------- -------
```

Please re-write Table 6, beginning on page 64, line 1, as follows:

Table 6 *ODN used with plasmid DNA*

Backbone	ODN code number	Sequence
S-ODN	1826	TCCATGACGTTCCTGACGTT (SEQ ID NO:51)
	1628	GGGGTCAACGTTGAGGGGGG (SEQ ID NO:52)
	1911	TCCAGGACTTTCCTCAGGTT (SEQ ID NO:53)
	1982	TCCAGGACTTCTCTCAGGTT (SEQ ID NO:54)
	2017	CCCCCCCCCCCCCCCCCCCC (SEQ ID NO:55)
O-ODN	2061	TCCATGACGTTCCTGACGTT (SEQ ID NO:56)
	2001	GGCGGCGGCGGCGGCGGCGG (SEQ ID NO:57)
SOS-ODN	1980	TCCATGACGTTCCTGACGTT (SEQ ID NO:58)
	1585	GGGGTCAACGTTGAGGGGGG (SEQ ID NO:59)
	1844	TCTCCCAGCGTGCGCCATAT (SEQ ID NO:60)
	1972	GGGGTCTGTGCTTTTGGGGGG (SEQ ID NO:61)
	2042	TCAGGGGTGGGGGGAACCTT (SEQ ID NO:62)
	1981	GGGGTTGACGTTTTGGGGGG (SEQ ID NO:63)
	2018	TCTAGCGTTTTTAGCGTTCC (SEQ ID NO:64)
	2021	TCGTCGTTGTCGTTGTCGTT (SEQ ID NO:65)
	2022	TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO:66)
	2023	TCGTCGTTGTCGTTTTGTCGTT(SEQ ID NO:67)

[*Note*: (SEQ ID NO:51-67, respectively)]

SOS-ODN had two S-linkages at the 5' end, five S-linkages at the 3' end, and O-linkages in between.

Three ODN used in this study were of the same murine-specific immunostimulatory sequence in three different backbones (1826, 2061 and 1980).

All ODN were synthesized by Hybridon (Milford, MA) or Operon (Alameda, CA). ODN were ethanol precipitated and resuspended in saline prior to use alone or as an additive to the plasmid DNA solution.

Please re-write Table 10 beginning on page 68, line 1, as follows:

Table 10

Inhibitory CpG motifs can block B cell proliferation induced by a stimulatory CpG motif

Oligonucleotide added	cpm
medium	194
1668 (TCCATGACGTTCCTGATGCT) (SEQ ID NO:68)	34,669
1668 + 1735 (GCGTTTTTTTTTGCG) (SEQ ID NO:69)	24,452
1720 (TCCATGAGCTTCCTGATGCT) (SEQ ID NO:70)	601
1720 + 1735	1109

Splenic B cells from a DBA/2 mouse were cultured at 5 X 10^4 cells/100 μl well in 96 well microtiter plates in RPMI as previously described (Krieg, *et al.,* 1995) with or without the indicated phosphorothioate modified oligonucleotides at a concentration of 60 ng/ml for 48 hr. The cells were then pulsed with ^{3}H thymidine, harvested, and the cpm determined by scintillation counting. The stimulatory CpG oligo 1668 was slightly but significantly inhibited by the inhibitory motifs in oligo 1735. The non CpG oligo 1720 is included as a negative control. [(SEQ ID NO:68-70, respectively).]

Please re-write Table 11, beginning on page 69, line 1, as follows:

Table 11

Inhibitory effects of "bad" CpG motifs on the "good" CpG Oligo 1619

Notes:

The sequence of oligo 1619 is TCCATGTCGTTCCTGATGCT (SEQ ID NO:71)

1949 has only 1 GCG at the 3' end, which has essentially no inhibitory activity

Oligonucleotide added	IL-12 in pg/ml
medium	0
1619 alone	6
1619 + 1949 (TCCATGTCGTTCCTGATGCG) (SEQ ID NO:72)	16
1619 + 1952 (TCCATGTCGTTCCGCGCGCG) (SEQ ID NO:73)	0
1619 + 1953 (TCCATGTCGTTCCTGCCGCT) (SEQ ID NO:74)	0
1619 + 1955 (GCGGCGGGCGGCGCGCGCCC) (SEQ ID NO:75)	0

Human PBMC were cultured in 96 well microtiter plates at 10^5/200µl for 24 hr in RPMI containing 10% autologous serum. Supernatants were collected at the end of the culture and tested for IL-12 by ELISA. All wells except the control (medium) contained 60 µg/ml of the stimulatory CpG oligodeoxynucleotide 1619; stimulatory (1949) and inhibitory (all other sequences have a strong inhibitory motif) oligos were added to the indicated wells at the same concentration at the beginning of culture. All oligos have unmodified backbones.

Please re-write Table 13 beginning on page 71, line 1, as follows:

Table 13 Identification of neutralizing CpG motifs which reduce the induction of cytokine secretion by a CpG-S motif in the same ODN (*cis*-neutralization)

ODN	sequence 5'-3'[1]	ODN-induced cytokine expression[2]		
		IL-6[2]	IL-12	IFN-γ
None		<5	206	898
1619	TCCATGTCGTTCCTGATGCT (SEQ ID NO:71)	1405	3130	4628
1952	. . . . .GCGCGCG (SEQ ID NO:73)	559	1615	2135
1953	. . . . CC. . . (SEQ ID NO:74)	577	1854	2000

[1]Dots in the sequence of ODN 1952 and 1953 indicate identity to ODN 1619; CpG dinucleotides are underlined for clarity. ODN without CpG-N or CpG-S motifs had little or no effect on cytokine production. The data shown are representative of 4 experiments.

[2]All cytokines are given in pg/ml; measured by ELISA on supernatants from DBA/2 spleen cells cultured in 96 well plates at 2 X 10^7 cells/ml for 24 hr with the indicated ODN at 30 μg/ml. Std. dev. of the triplicate wells was <7%. None of the ODN induced significant amounts of IL-5

Please re-write Table 14 beginning on page 72, line 1, as follows:

Table 14 Inhibition of CpG-induced cytokine secretion by ODN containing CpG-N motifs

ODN	sequence 5'-3'	IL-12 secretion[1]	CpG-S-induced IL-12 secretion[2]
none		268	5453
1895	GCGCGCGCGCGCGCGCGCGC (SEQ ID NO:76)	123	2719
1896	CCGGCCGGCCGGCCGGCCGG (SEQ ID NO:77)	292	2740
1955	GCGGCGGGCGGCGCGCGCCC (SEQ ID NO:75)	270	2539
2037	TCCATGCCGTTCCTGCCGTT (SEQ ID NO:78)	423	2847

[1]BALB/c spleen cells were cultured in 96 well plates at 2 X 10^7 cells/ml with the indicated ODN for 24 hr and then the supernatants were assayed for IL-12 by ELISA (pg/ml).

[2]Cells were set up the same as in [1] except that IL-12 secretion was induced by the addition of the CpG ODN 1619 [(TCCATGACGTTCCTGATGCT)] (TCCATGTCGTTCCTGATGCT) (SEQ ID NO: 71) at 30 µg/ml. The data shown are representative of 5 experiments.

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